Relationship Between Hydrolytic Rancidity, Oil Concentration, and Esterase Activity in Rice Bran

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ABSTRACT

Hydrolytic rancidity restricts the utilization of rice bran, reducing its potential value. In the present study, three groups of eight rice cultivars each displaying different levels of oil concentration (high, medium, and low) were cultivated in 1999 and 2000 under field conditions and evaluated for oil content, hydrolytic rancidity, and esterase activity in the bran fraction. Genotype effects were statistically significant for all measured traits ($P < 0.05$), whereas environment (year) was nonsignificant. Hydrolytic rancidity was strongly correlated with esterase activity ($r = 0.89$***), but not with oil concentration ($r = -0.01$). A wide variation was found for both hydrolytic rancidity and esterase activity, which ranged from 6.8 to 56.0 mg of C8:0/g of bran (CV = 49.1%) and from 4.3 to 22.8 mg of C8:0/g of bran (CV = 34.3%), respectively. Red bran displayed the lowest values for both hydrolytic rancidity (mean = 10.2 mg of C8:0/g of bran) and esterase activity (mean = 5.4 mg of C8:0/g of bran). Apparently, the low values for hydrolytic rancidity were related to the inhibition effect of bran tannins on lipase activity. In conclusion, cultivar variation was detected for both hydrolytic rancidity and esterase activity in the studied genotypes, esterase activity being the principal factor explaining the variation found for the former trait. Therefore, it may be possible to create new cultivars with increased stability against hydrolytic rancidity by selecting for lower esterase activity.

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Rice (*Oryza sativa* L.) bran is a by-product of the rice milling process and a valuable source of various phytochemicals such as phenolics, tocotrienols, and γ-oryzanol. However, as a food ingredient, its use is severely limited by its high susceptibility to developing hydrolytic rancidity. During the milling process, rice bran lipids come into contact with lipases that rapidly hydrolyze the ester bonds of triacylglycerol (esterase activity), releasing fatty acids (known as free fatty acids), and glycerol (Ramezanzadeh et al 1999). The free fatty acids increase acidity, generate unacceptable functional properties, and produce undesirable organoleptic characteristics. As a consequence, the bran becomes unsuitable for human consumption or for production of edible oil with acceptable quality (Barnes and Galliard 1991). Although the lipolytic process can be inhibited by deactivating lipases through the use of various stabilization methods (Malekian et al 2000), such procedures are only economically justifiable for large-scale operations, which are not common in rice processing (McCaskill and Zhang 1999). Therefore, a cost-effective alternative is desired to reduce the susceptibility of rice bran to hydrolytic rancidity. The use of breeding techniques could be effective in increasing the stability of rice bran against lipid hydrolysis if genetic differences between cultivars exist for this trait. Tsuzuki et al (1994) observed cultivar variation for esterase activity in rice bran. Significant variation (17.3–27.4%) in rice bran oil content has been reported by Goffman et al (2002). By monitoring the hydrolytic degradation of bran lipids during storage in two rice cultivars, Goffman and Bergman (2003) also found significant differences in esterase activity, suggesting that lipase (esterase) activity may be an important factor determining the intensity of the hydrolytic degradation of bran lipids, whereas bran oil concentration may not be as significant as lipase activity in this process. To determine the relative significance of lipase activity and oil concentration to the intensity of hydrolytic process in rice bran, an experiment including genotypes showing different oil concentrations in the bran is required. In the present study, 24 rice cultivars differing in oil content were evaluated for hydrolytic rancidity and esterase activity in the bran.

MATERIALS AND METHODS

Plant Material

Three groups of eight rice (*Oryza sativa* L.) cultivars displaying low (16–20%), medium (20–24%), and high (24–28%) bran oil content were used in this study. The genotypes were selected from a previous study which included more than 200 rice accessions (Goffman et al 2002). The cultivars were grown under field conditions in Beaumont, TX, using cultural management practices common for the region. The plants were cultivated in single plots, arranged in a completely randomized design. The plots consisted of six rows, 3.5 m long, spaced 15 cm apart. The within-row spacing was ≈10 cm. The plots were kept continuously flooded at ≈10 cm of standing water. At maturity, the plants were threshed by hand, the grains were dehulled, and all broken, diseased, and immature kernels were removed. Dehulled kernels (≈50 g) were milled using a McGill mill #1 for 30 sec with an 858 g weight in position 12 and 6 for long and medium grain types, respectively. The bran fraction was collected and sieved through a 840-µm sieve. Bran samples were conserved in a freezer (–20°C) under nitrogen until analysis. Surface lipid content was determined by refluxing 5 g of milled rice with petroleum ether in a Goldfish extraction apparatus for 30 min. The solvent was collected and evaporated, percent surface lipid content was calculated as the mass of the extracted lipid divided by the beginning total milled rice mass. This measurement was used to ensure that all samples were milled within a similar range in degree of milling (i.e., <0.5% surface lipid content).

Determination of Hydrolytic Rancidity

The hydrolytic deterioration of rice bran lipids was estimated as the accumulation of free fatty acids (FFA) in the bran after 48 hr of storage. Preliminary analysis (not reported) indicated this storage period was optimal for detecting differences in the hydrolytic rancidity of bran lipids between cultivars. Fresh rice bran (≈200 mg) was stored in an incubator for 48 hr at 35°C in 5-mL sealed polypropylene test tubes. After incubation, the accumulation of FFA was measured according to the method of Kwon and Rhee (1986) with minor modifications and using a caprylic acid

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(C8:0) as external standard. Isooctane (3 mL) was added to each tube. The samples were extracted for 5 min using a shaker (Innova 2000, New Brunswick Scientific, NJ) at 250 rpm. The tubes were then centrifuged for 5 min at 4,000 rpm with a centrifuge (CR 4.12, Jouan, Winchester, VA) and 2 mL of the supernatant were pipetted into a new tube containing 1 mL of reagent (3% v/v pyridine in a 5% w/v aqueous cupric acetate solution). The mixtures were mixed using the shaker at 250 rpm for 5 min. After sedimentation of the aqueous phase, an aliquot of the isooctane phase was measured at 715 nm. Hydrolytic rancidity was expressed as mg of C8:0 equivalents/g, dry weight, of rice bran. Analysis was performed with two replicates.

Analysis of Bran Oil Content

Rice bran (≈400 mg) was dried for 2 hr at 105°C for moisture content determination. The dried rice bran was further used for measuring oil content by Soxhlet extraction with light petroleum ether (boiling point 40°C). Extraction time was 12 hr. Previous work (Chen and Bergman 2002) indicates that rice accessions with a range in milling quality will vary in bran starch content. Poor milling quality lines will have greater starch content while those with superior whole grain yields have lower starch content. These varying levels of starch will confound data that is expressed on a bran weight basis. To eliminate the effect of different milling qualities on the oil content, the values were corrected to an equivalent of 15% w/w starch content in the bran, using the formula

$$\text{Corrected oil content (\%w/w)} = \text{Oil content as is (\%w/w)} \times (100\%-15\%)/(100\%-\%\ \text{of starch of the sample})$$

Bran oil content was expressed as %w/w (dry weight basis). Analysis was made in duplicate.

Starch Content Determination

A method by McClearly et al (1997) for starch content was modified for rice bran analysis (Chen and Bergman, *personal communication*). Rice bran (50 mg) was weighed into a test tube and wet with 200 µL of aqueous ethanol (80% v/v). Dimethyl sulphoxide (2 mL of DMSO) was added immediately, and the tubes were stirred with a vortex mixer and placed in boiling water for 5 min. After that, 3 mL of thermostable α-amylase (100 U/mL) in a 4-morpholinepropanesulfonic acid sodium salt (MOPS) buffer (50 m*M*, pH 7.0) containing calcium chloride (5 m*M*) and sodium azide (0.02% w/v) were immediately added. The tubes were vigorously stirred on a mixer three times while incubated in boiling water for 6 min. The samples were then transferred to a bath at 50°C, and 4 mL of sodium acetate buffer (200 m*M*, pH 4.5) was added, followed by 0.1 mL of amyloglucosidase (200 U/mL). They were then stirred and incubated for 30 min at 50°C. After incubation, the samples were adjusted to 10 mL volume and mixed thoroughly. An aliquot of 200 µL of this solution was centrifuged at 3,000 rpm for 10 min and diluted to 600 µL with distilled water. Glucose determination reagent (3 mL) (12,000 U/L of glucose oxidase, 650 U/L of peroxidase, and 0.4 m*M* 4-aminoantipyrine) were added, and the samples were held at 50°C for 20 min. The absorbance at 510 nm was measured for each sample and starch contents were calculated using a calibration curve developed with glucose standards. Starch content was expressed as %w/w (dry weight basis). Analysis was performed with two replicates.

Esterase Activity

Esterase activity was determined in duplicate after extracting esterases from fresh bran samples using Tween 20 as the enzyme-substrate. Extraction media, incubation temperature, and enzyme reaction time were selected according to the optimal conditions established by Prabhu et al (1999) for rice bran lipase. Esterases were extracted from ≈200 mg of rice bran with 2 mL of 50 m*M* potassium phosphate buffer (pH 7.2) for 1 hr using a shaker at 250 rpm. The samples were then centrifuged for 5 min at 4,000 rpm and 1 mL of the aqueous layer was pipetted into a tube containing an aqueous Tween 20 solution (30 mg/mL). The samples were incubated at 35°C for 18 hr. After that, the released FFA were extracted with 3.5 mL of isooctane and measured as indicated above. Esterase activity was expressed as mg of C8:0 equivalents/g of dry weight rice bran.

Statistical Analysis

The data was first subjected to a one-way nonparametric analysis of variance using the test developed by Kruskal and Wallis (1952). The cultivars were then separated into groups and a factorial analysis of variance was performed with year and group considered as factors. Group effect was further partitioned into variation due to comparisons among groups. All statistical analyses were performed with statistical software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Table I shows the chi-square approximations of the nonparametric analysis of variance for oil content, hydrolytic rancidity, and esterase activity. Genotype effects were highly statistically significant for all measured traits ($P < 0.05$). Environment (year) did not significantly affect the traits analyzed. The chi-square approximation values due to genetic effects overwhelmed those caused by seasonal influences, indicating that most of the variation for these traits was due to genetic differences among the cultivars. But genotype effects may be overestimated because the experimental design used does not allow estimation of genotype-year interactions.

Hydrolytic rancidity was highly significantly correlated with esterase activity ($r = 0.89$***) but not with bran oil concentration ($r = -0.01$) (Figs. 1 and 2, respectively). That indicates that the intensity of the lipolytic process is strongly related to lipase activity but not to the oil content of the bran. Our data agreed with the results obtained earlier (Goffman and Bergman 2003), which suggested lipase activity was the primary factor determining the intensity of the hydrolytic deterioration of rice bran oil.

In Fig. 2, the cultivars were separated into groups according to their oil concentration (A, B, and C for low-, medium-, and high-oil content, respectively). The A group was further divided into two subgroups based on differences in the susceptibility to rancidity, which interestingly were associated with the bran color (A_1, low hydrolytic rancidity values, red bran; and A_2, high hydrolytic rancidity values, light and speckled brown bran cultivars). A factorial analysis of variance was performed considering group and year as factors (Table II). Year was only statistically significant for oil content ($P < 0.01$). The interactions year × all groups and year with all group comparisons were only significant for oil content, except for the interaction year × [A_1 vs. A_2, B, and C], which was nonsignificant. The results indicate that hydrolytic rancidity and esterase activity are not affected by seasonal (year) effects or their interactions. Group significantly differed for all analyzed traits at $P = 0.001$. Red bran genotypes (A_1) strongly differed from light and speckled brown bran genotypes for hydrolytic rancidity and esterase activity ($P < 0.001$). Within the light and speckled brown bran genotypes, the low-oil cultivars showed significant differences at $P = 0.001$ for hydrolytic rancidity and esterase activity when compared with the medium- and high-oil cultivars. Medium- and high-oil cultivars did not differ for hydrolytic rancidity and esterase activity.

The means over two years for oil content, hydrolytic rancidity, and esterase activity are presented in Table III. A wide variation was found for both hydrolytic rancidity and esterase activity: from 6.8 to 56.0 mg of C8:0/g of bran (CV = 49.1%) and from 4.3 to 22.8 mg of C8:0/g of bran (CV = 34.3%), respectively. Red bran cultivars showed the lowest values for both hydrolytic rancidity

(mean 10.2 mg of C8:0/g of bran) and esterase activity (mean 5.4 mg of C8:0/g of bran). Goffman and Bergman (2002) found that red bran genotypes on average show 50-fold more tannin concentration in the bran than light and speckled brown bran cultivars. In vivo and in vitro studies showed a reduction of lipase activity in the presence of tannins (Horigome et al 1988; Longstaff and McNab 1991). Therefore, the low values for hydrolytic rancidity observed in the red bran cultivars may be related to the inhibitory effect of tannins on bran lipase activity. But further investigations using purified rice bran lipase and different concetrations of rice bran tannins are required to confirm this hypothesis. Low-oil cultivars with nonred bran surprisingly showed the highest values for esterase activity (mean 20.2 mg of C8:0/g of bran) and thus for hydrolytic rancidity. It can be speculated that the higher esterase activity found in those cultivars constitutes an ecological advantage for the rapid and efficient production of carbohydrates by lipid catabolism, a process required during germination. Further research is nevertheless needed to determine whether this association holds across other germplasm and, if so, to elucidate the biochemical and genetic reasons behind the association.

TABLE I
Chi-Square Approximations (χ^2) of Nonparametric Analysis of Variance for Oil Content, Hydrolytic Rancidity (HR), and Esterase Activity (EA)

Source	df	Oil[a]	HR[b]	EA[b]
Genotype	23	41.36*[c]	36.97*	40.70*
Year	1	0.23ns	0.27ns	0.94ns

[a] Oil content expressed as %w/w of bran (15% starch content basis).
[b] Expressed as mg of C8:0 equivalent/g of bran.
[c] *, Significant at $P = 0.05$ (Kruskal-Wallis test); ns, nonsignificant.

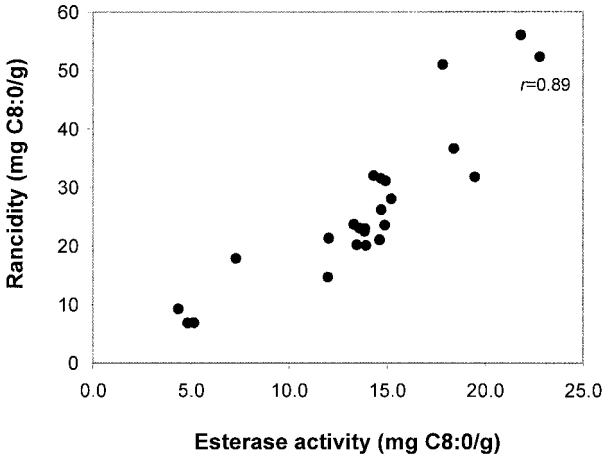

Fig. 1. Scatter plot of esterase activity (mg of C8:0 equivalents/g of bran) vs. hydrolytic rancidity (mg of C8:0 equivalents/g of bran) in 24 rice accessions (means over two seasons).

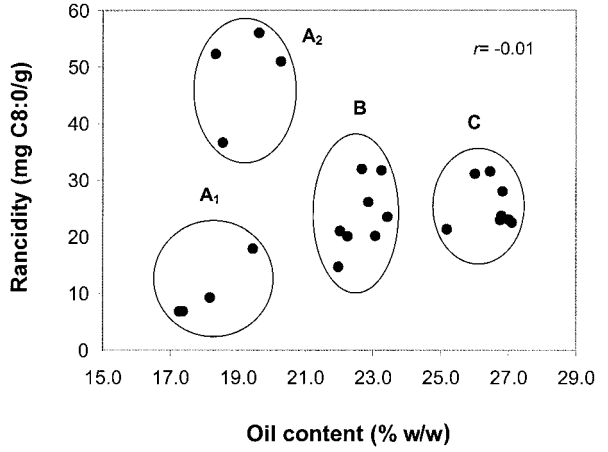

Fig. 2. Scatter plot of oil content (% w/w bran) vs. hydrolytic rancidity (mg of C8:0 equivalents/g of bran) in 24 rice accessions (means over two seasons). A_1, low-oil content, red bran; A_2, low-oil content, nonred bran; B, medium-oil content, nonred bran; C, high-oil content, nonred cultivars.

CONCLUSIONS

Cultivar variation was detected for both hydrolytic rancidity and esterase activity, which suggests that it is possible to reduce the susceptibility of rice bran to hydrolytic deterioration by using classical breeding techniques. Moreover, it was not affected by

TABLE II
Analysis of Variance for Oil Content, Hydrolytic Rancidity (HR), and Esterase Activity (EA)

		Mean Square Values		
Source	df	Oil[a]	HR[b]	EA[b]
Year	1	7.74**[c]	35.81ns	7.49ns
All groups[d]	3	167.74***	2,101.25***	301.85***
A_1 vs. A_2, B and C	1	198.83***	2,467.58***	677.60***
A_2 vs. B and C	1	187.30***	3,808.92***	223.62***
B vs. C	1	117.09***	27.24ns	4.32ns
Year × all groups	3	12.05***	70.32ns	7.48ns
Year × [A_1 vs. A_2, B and C]	1	2.31ns	0.01ns	11.63ns
Year × [A_2 vs. B and C]	1	10.30***	9.92ns	2.44ns
Year × [B vs. C]	1	23.54***	201.03ns	8.37ns
Error	40[e]	0.95	71.34	4.54

[a] Expressed as %w/w of bran (15% starch content basis).
[b] Expressed as mg of C8:0 equivalents/g of bran.
[c] *, Significant at $P = 0.05$ (Kruskal-Wallis test); ns, nonsignificant.
[d] Groups A_1, low-oil content, red bran; A_2, low-oil content, nonred bran; B, medium-oil content, nonred bran; C, high-oil content, nonred bran cultivars.
[e] Value of df reduced because group A was split into two subgroups.

TABLE III
Means Over Two Seasons for Oil Content, Hydrolytic Rancidity (HR), and Esterase Activity (EA) of 24 Studied Rice Accessions

Oil Class/Cultivar	Bran Color	Oil (%)[a]	HR[b]	EA[b]
Low oil content				
Banjul	Red	17.3	6.8	4.8
Chokoto	Red	17.4	6.9	5.1
Kun Shan Wu Shan	Red	18.2	9.3	4.3
Achhame	Red	19.4	17.9	7.3
WC 756	Light brown	18.3	52.3	22.8
Japonesito	Light brown	18.5	36.6	18.4
WIR 605	Light brown	20.3	51.0	17.8
Primorsk 6	Speckled brown	19.6	56.0	21.8
Medium oil content				
Sadri Type	Light brown	22.0	21.0	14.6
Teqing	Light brown	22.0	14.7	12.0
Dom Sofid	Light brown	22.2	20.1	13.9
L144	Light brown	22.7	32.0	14.3
Lacrosse	Light brown	22.9	26.1	14.7
XB-1	Light brown	23.1	20.2	13.5
Daw Dam	Light brown	23.2	31.7	19.5
Lulks	Speckled brown	23.4	23.5	14.9
High oil content				
Yodanya	Light brown	25.2	21.3	12.0
L-201	Light brown	26.0	31.1	14.9
Early No. 2	Light brown	26.5	31.6	14.7
Chin Chin	Speckled brown	26.8	23.6	13.3
IR 66-103-2	Light brown	26.8	22.9	13.9
L205	Light brown	26.8	28.0	15.2
Goolarah	Light brown	27.0	23.0	13.6
Newrex	Light brown	27.1	22.5	13.9
LSD[c]		3.2	17.7	4.0

[a] Expressed as %w/w of bran (15% starch content basis).
[b] Expressed as mg of C8:0 equivalents/g of bran.
[c] Least significant difference ($P < 0.05$).

season and thus could be evaluated easily. Esterase activity was a principal factor explaining most of the variation for hydrolytic rancidity between the studied cultivars. By selecting for lower esterase activity, it appears possible to create new cultivars with increased stability against hydrolytic rancidity. Red bran cultivars seem to be an interesting genetic group to be considered for finding low susceptibility to rancidity. If the tannin content is the main cause of the low values for esterase activity in the red bran cultivars, it will be interesting to include genotypes with other bran colors (dark brown and purple) in screening programs searching for low esterase activity.

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